

In Vitro Evaluation of a New Quinolone Antibacterial

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The activity of R-802, a quinolone antibacterial agent, was studied in vitro and found to be active against *Enterobacteriaceae*; less than 4 μg of drug per ml was required to inhibit most isolates. The majority of *Pseudomonas aeruginosa* grew in a concentration of 256 μg of R-802 per ml when studied in broth against an inoculum of 10^8 organisms per ml.

The present studies were performed to assess the in vitro antibacterial activity of R-802, a synthetic quinolone antibacterial agent similar in structure to nalidixic and oxolinic acids (see Fig. 1)(Riker Laboratories, Inc.).

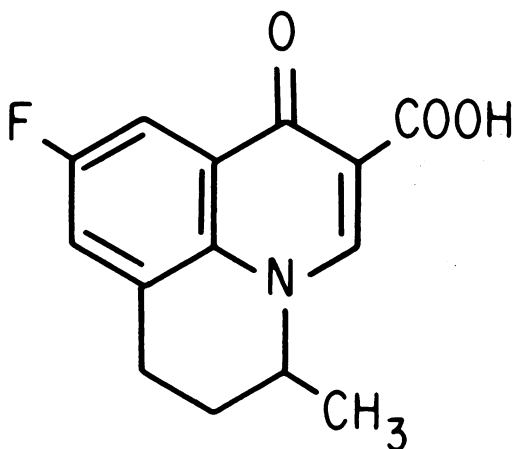


FIG. 1. Structure of R-802. Chemically the compound is 6,7-dihydro-9-fluoro-5-methyl-1-oxo-1H,5H-benzo (ij) quinolizine-2-carboxylic acid.

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Antibacterial activity was determined by antibiotic dilution methods performed in nutrient (Difco) and Mueller-Hinton (Difco) agar and broth. Studies in agar were performed by use of a Steers replicator device (3). The methods for performing the agar and broth dilution studies have been described previously from this laboratory (1, 2). A total of 150 isolates of *Enterobacteriaceae* and 30 strains of *Pseudomonas aeruginosa* were studied.

The cumulative percentage of 30 isolates each of *Escherichia coli*, *Enterobacter*, *Klebsiella*, indole-positive *Proteus*, *Proteus mirabilis*, and *P. aeruginosa* susceptible to increasing concentrations of R-802 is shown in Fig. 2 and 3, which also summarize the effects of medium and inoculum size on the antibacterial activity of this compound in agar. The results of these studies performed in agar demonstrate that R-802 was active against *Enterobacteriaceae*, and all 150 isolates were inhibited by 16 μg or less of the drug per ml in both media. In

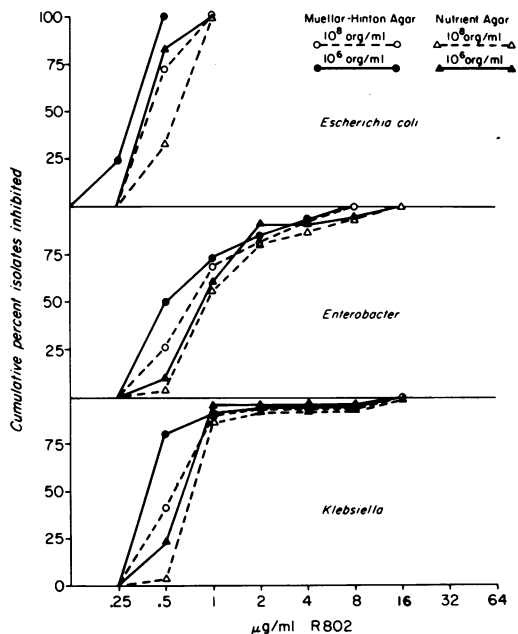


FIG. 2. Cumulative percentage of 30 isolates each of *E. coli*, *Enterobacter*, and *Klebsiella* inhibited by increasing concentrations of R-802 tested in agar medium with bacterial inocula of two different sizes.

addition, increasing the size of the inoculum 100-fold exerted little effect on the overall activity of R-802 in agar. Although strains of *P. aeruginosa* were more resistant in agar than were *Enterobacteriaceae*, all 30 isolates were inhibited by 128 μg or less of R-802 per ml

(feasible urine levels when 400 mg of drug is administered four times a day).

Figures 4 to 7 summarize the inhibitory (minimal inhibitory concentration) and bactericidal (minimal bactericidal concentration) activities of R-802 against *Enterobacteriaceae* and *Pseudomonas* tested in broth medium with two inoc-

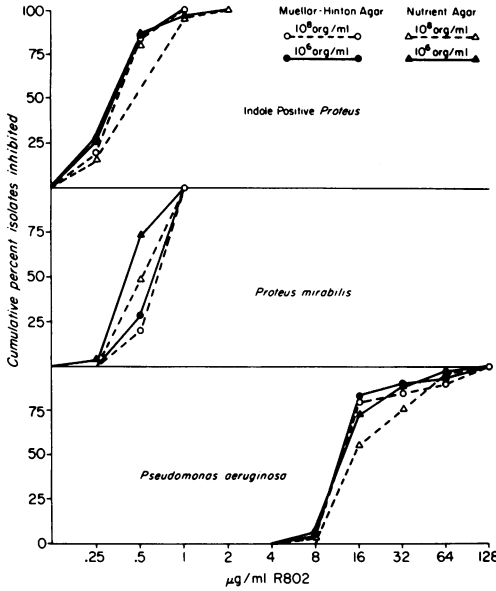


FIG. 3. Cumulative percentage of 30 isolates each of indole-positive *Proteus*, *P. mirabilis*, and *P. aeruginosa* inhibited by increasing concentrations of R-802 tested in agar medium with bacterial inocula of two different sizes.

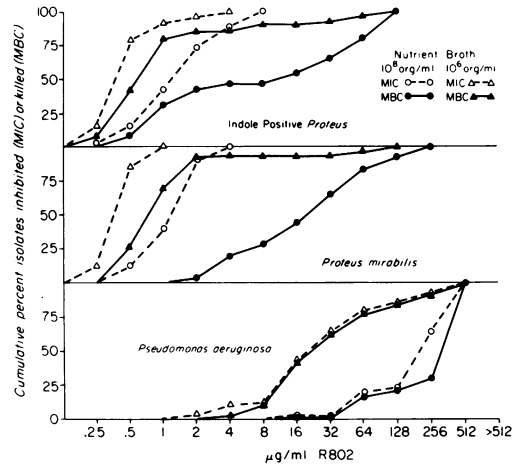


FIG. 5. Cumulative percentage of 30 isolates each of indole-positive *Proteus*, *P. mirabilis*, and *P. aeruginosa* inhibited (MIC, minimal inhibitory concentration) or killed (MBC, minimal bactericidal concentration) by increasing concentrations of R-802 tested in nutrient broth medium with bacterial inocula of two different sizes.

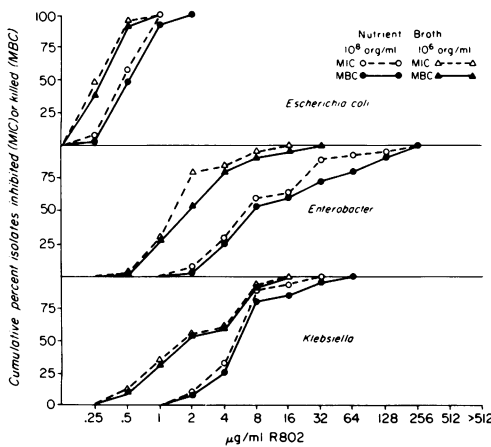


FIG. 4. Cumulative percentage of 30 isolates each of *E. coli*, *Enterobacter*, and *Klebsiella* inhibited (MIC, minimal inhibitory concentration) or killed (MBC, minimal bactericidal concentration) by increasing concentrations of R-802 tested in nutrient broth medium with bacterial inocula of two different sizes.

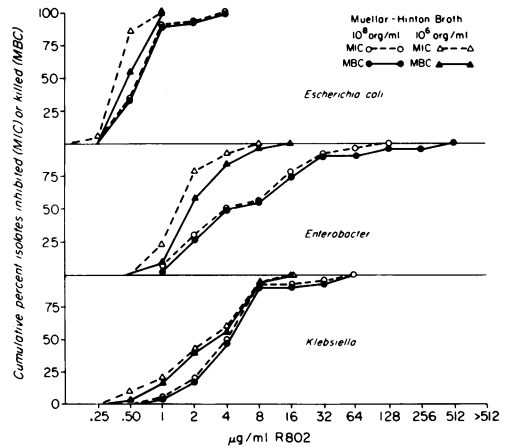


FIG. 6. Cumulative percentage of 30 isolates each of *E. coli*, *Enterobacter*, and *Klebsiella* inhibited (MIC, minimal inhibitory concentration) or killed (MBC, minimal bactericidal concentration) by increasing concentrations of R-802 tested in Mueller-Hinton broth medium with bacterial inocula of two different sizes.

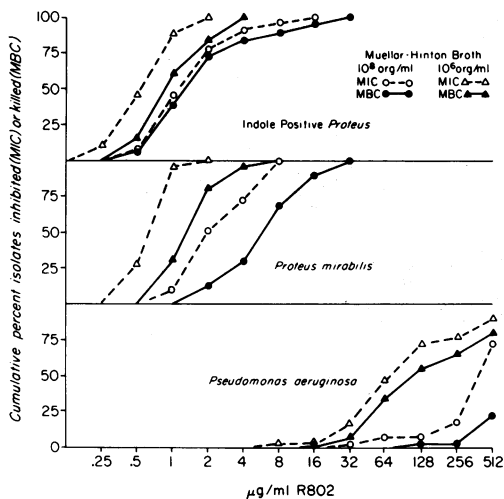


FIG. 7. Cumulative percentage of 30 isolates each of indole-positive *Proteus*, *P. mirabilis*, and *P. aeruginosa* inhibited (MIC, minimal inhibitory concentration) or killed (MBC, minimal bactericidal concentration) by increasing concentrations of R-802 tested in Mueller-Hinton broth medium with bacterial inocula of two different sizes.

ulum sizes of bacterial cells. Against all genera, R-802 demonstrated lesser activity in broth than in agar. For example, whereas approximately 90% of 150 isolates of *Enterobacteriaceae* had been inhibited by 1 µg or less of R-802 per ml when tested in agar, 16 µg/ml was needed to inhibit a comparable number of strains in broth medium. Moreover, although there was parallel activity between inhibitory and bactericidal concentrations of R-802 against most genera, isolates of *P. mirabilis*, indole-positive *Proteus* sp., and *P. aeruginosa* survived in high concentrations of

R-802. The decreased bactericidal activity was most apparent when tests were performed in Mueller-Hinton broth with the larger inoculum size of bacterial cells. For example, whereas 100% of *P. aeruginosa* was both inhibited and killed by 512 µg of the drug per ml when tested in nutrient broth against 10⁸ organisms per ml, only 75 and 25% of these isolates were inhibited and killed, respectively, in Mueller-Hinton broth.

The results of these studies indicate that R-802 is active in vitro against *Enterobacteriaceae*. In nutrient broth with a large inoculum size of bacterial cells (10⁸ organisms per ml), less than 4 µg of R-802 per ml was required to inhibit most *Enterobacteriaceae*. Moreover, less than 1 µg/ml was necessary for inhibition when an inoculum of 10⁶ organisms per ml was used. Although most isolates of *Pseudomonas* were susceptible to feasible urine levels when studied in agar, the majority of strains grew in concentrations of 256 µg of R-802 per ml when studied in broth against the larger inoculum size of bacterial cells. Because of these encouraging in vitro results, a double-blind clinical study comparing two dosage forms of R-802 in women with bacteriuria has been initiated.

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